CHAPTER 15

RE√EAL FOR AFLATOXIN TEST KIT

15.1 GENERAL INFORMATION

The Re√eal for Aflatoxin kit from Neogen is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The test provides a qualitative determination of the presence or absence of aflatoxin at a 20 ppb threshold level. Using a aflatoxin-antibody particle complex coated test strip, the inspector is able to determine the presence or absence of aflatoxin in the sample by the formation, or lack thereof, of a visible line on the test strip.

15.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the Re√eal for Aflatoxin test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (Reagent grade or better) and 30 percent water.

a. Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

b. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

c. Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

15.3 EXTRACTION PROCEDURES

a. Transfer 50 grams of ground sample into an extraction mixing jar.

b. Add 100 ml of the (70/30) methanol/water extraction solvent.

c. Cover the extraction jar and blend on high speed for 1 minute.

d. Allow the sample to settle, filter the extract through a filter syringe or filter paper, and place into a sample jar labeled with the sample identification.

e. After collecting the filtrate dispose of the filter and ground material and set the filtrate aside for testing.

15.4 TEST PROCEDURES

a. Sample Analysis.

(1) Remove the appropriate number of sample cups and place in a sample cup rack.

(2) Using a pipettor and new tip, add 200 microliters (µl) of sample diluent to a sample cup.

(3) Using a new tip, add 200 µl of the clear portion of the filtered sample extract to the sample cup. (This is a 1:1 dilution.)

(4) Mix the solution by pipetting up and down 3 times.

(5) Place a new aflatoxin test strip with the sample end down into the sample cup.

(6) Allow the strip to develop in the sample cup for 3 minutes.

(7) At the end of the 3 minute development period, remove the strip from the sample cup and read the results visually against a white background or with Neogen's Re√eal Accuscan Reader.

b. Interpreting the Lateral Flow Test Strip by Visual Analysis.

Development of a line in the Control Zone of the test strip within 3 minutes indicates that the strip has functioned properly. Any strip that does not develop a line in the Control Zone should be discarded. A second preparation of the extract (using a fresh 1:1 dilution) should be made and tested using another strip.

(1) Negative Result (≤ 20 ppb).

If a line forms in the test zone and another line forms in the control zone within 3 minutes (resulting in 2 visible lines on the test strip), the sample is considered negative (equal to or less than 20 ppb) for aflatoxin. A negative sample may be determined before the full 3 minute development if 2 lines are visible on the strip.

(2) Positive Result (> 20 ppb).

If after the full 3 minutes there is no visible line in the Test Zone, but a visible line exists in the Control Zone, the sample is considered positive (exceeding 20 ppb) for aflatoxin

c. Reading Lateral Flow Test Strip Results with the Re√eal Accuscan Reader.

The strips can be read using Neogen's Reveal Accuscan Reader that incorporates a PDA with a Reveal Accuscan Lateral Flow optical reader. Using the reader helps eliminate subjectivity by interpreting and storing sample results. Operating instructions are included with the optional reader.

15.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report results on the pan ticket and inspection log as being equal to or less than 20 ppb (≤ 20 ppb), or as exceeding 20 ppb (> 20 ppb), as applicable.

b. Certify results as being equal to or less than 20 ppb or exceeding 20 ppb, as applicable.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

15.6 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used extraction mixing jars, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used extraction mixing jars and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used test strips and pipettes for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

15.7 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), dispose of any remaining liquid filtrate in the chemical waste container. Discard the sample slurry (ground material) into a plastic garbage bag for disposal.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the slurry (ground portion) remaining in the sample extraction jar must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, pour approximately 50 ml of bleach solution into the sample extraction jar and shake to mix with the sample slurry. After the slurry and bleach solution separate, handle the bleach rinse filtrate as a non-hazardous solution and dispose of by pouring the liquid down the down. Discard the sample slurry (ground portion) paper into a plastic garbage bag for disposal.

15.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

(1) 25 aflatoxin test strips.

(2) 25 sample cups.

(3) 1 bottle of sample diluent.

b. Materials Required but not Provided:

(1) Timer (3 minute capacity).

(2) 200 µl pipettor (single or multi-channel) with tips.

(3) Sample cup holder.

(4) Balance.

(5) Sample Grinder.

(6) Methanol - ACS grade or better.

(7) Deionized or Distilled Water.

(8) Blender with mixing jars.

(9) Filter Paper or Coffee Filters.

15.9 STORAGE CONDITIONS

a. Storage Conditions.

(1) Store kit components at room temperature (18°- 30°C, 64°- 86°F) at room temperature when not in use to assure full shelf life. Test strips must remain capped in the stay-dry tube before use to assure optimal performance.

(2) Do not freeze test kit components.

b. Precautions.

(1) Do not use kit components beyond expiration date.

(2) Test strip development times other than those specified may give inaccurate results.